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PROBIOTICS: EFFECT ON SHRIMP POST LARVAE GROWTH OF P. MONODON

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ABSTRACT

Shrimp aquaculture, constantly requires new techniques in order to increase culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms with delivering living bacterial cells to the gut ecosystem of humans and other animals. Feeding and new practices in hatchery farming usually play an important role in aquaculture, and the addition of various additives to a balanced feed formula to achieve better growth is a common practice of many fish and shrimp feed manufacturers and farmers. Important tools that could lead to a higher quality and greater quantity of products.

KEYWORDS: P. Monodon, Probiotics, Growth and Hatchery.

1. INTRODUCTION

"Probiotic bacteria or beneficial bacteria", cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal balance and health of the host. The first probiotic discovered long time ago was Lactobacillus sp., the lactic acid producing bacteria. The traditional methods of prawn culture have been practiced for centuries in some Asian countries. In recent years the system of prawn farming has got a bright impetus and is well established in different parts of the world. Now a day's prawn culture in India has been reached at a significant level (1). Keeping in view of the present demand for larval rearing, culture, nutritional requirement, production and marketing potentialities of certain brackish water species such as *Penaeus monodon, Penaeusindicus, Penaeusmerguiensis* etc., nowadays a number of shallow brackish water bodies in the country were brought under scientific farming.

Shrimp farming is very important in aquaculture today. From last 20 years Shrimp aquaculture in the world developed remarkably. In 1997, more than 941 000 metric tonnes of farmed shrimp valued over US$ 6.07 billion were produced. India is one of the top ten fish producing countries of the world with a coastline about 5700 km on the mainland and about 7500 km including the two island territories and the total brackish water area along this coastline is estimated to be about 11,90,900 hectares. These areas are ideal for brackish water shrimp/prawn culture (2).

Unfortunately the main bottleneck in the culturing of these penaeid shrimps is the limitation of brackish water resources and also management skills (3). Presently, the total World production of penaeid shrimps is 1,61,8425 Mt. Furthermore, a remarkable achievement was made in the hatchery management and larval rearing technique of penaeid species especially *Penaeusmonodon, P. indicus, P. japonicus* and *P. vannamei* through adoption of scientific principles (5). MPEDA estimated that out of 12.38 lakh hectares of potential area for shrimp culture, only 1.42 lakh hectares i.e. 11.92% is currently used in India (6).

The country again suffered a major setback again in brackish water aquaculture when the most valuable shrimp crops meant for export were lost in almost all maritime states particularly Andhra Pradesh. This was due to white spot syndrome and due to this a large number of farms were closed and farmers gave up shrimp farming which leads to decline in foreign exchange through shrimp farming (7).

There are a wide variety of vaccines available for other sectors but a very few in the fisheries sector. However the indiscriminate use of antibiotics has led to increased antibiotic resistance and problem of tissue residues and trade issues. Therefore alternative ways are needed to overcome from the present problem. It might be of assistance to use of "probiotics" in the treatment (8). Probiotic bacteria are generally called the bacteria which can improve the water quality of aquaculture, and (or) inhibit the pathogens in the water thereby increasing production. It includes photosynthetic bacteria, such as l*actobacillus, actinomycetes, nitrobacteria, denitrifying* bacteria, *bifido bacterium*, yeast etc (9).

The success of any sustainable aquaculture system depends mainly on the production of disease free healthy post larvae from hatcheries (10). It also greatly depends on nutritious, eco-friendly and economically viable management strategies. The World Aquaculture production has been increasing linearly for more than a decade and the rate of increase has remained fairly constant (11).

2. MATERIALS AND METHODS

2.1 Probiotic samples:

Ten probiotic product samples used for marine shrimp cultivation available in India were purchased from a local aquaculture product retailer (distributor) as shown in Table 1. All samples were stored at 4°C before use

DOC	WP/hectare(A1)	FP/kg feed(A2)	WP+FP(A3)
15-20	2kg	10gm	2kg+5gm
21-30	2kg	10gm	2kg+5gm
31-40	2kg	10 gm	2kg+5gm
41-50	2kg	10 gm	2kg+5gm
51-60	2kg	10 gm	2kg+5gm
61-70	2kg	10 gm	2kg+5gm
71-80	2kg	10gm	2kg+5gm

81-90	2kg	10gm	2kg+5gm
91-100	2kg	10gm	2kg+5gm
101-110	2kg	10gm	2kg+5gm
111-125	2kg	10gm	2kg+5gm

Table- 1 Dosage probiotics at different DOC

2.2 Hatchery production of shrimp post larvae:

2.1.1 Selection of Brood stock:

Hatchery success depends to a large extent on the quality of broodstock selected for maturation. Every effort should be made to ensure that only large, productive, healthy, disease-free shrimp. For the present study *P. monodon* brood stock caught from costal belt of Bay of Bengal, Andhra Pradesh. To check the viral infections preliminarily, a piece of pleopod (or telson) is cut from each shrimp (the place where the cut was made was disinfected with pure liquid povidone PVP iodine) and preserved in 90% alcohol in a small bottle or tube. This was then sent to a PCR laboratory to check for WSSV. MBV can also be checked from this by PCR.

The present study was carried out in Krishna hatchery, located at Bapatla Guntur district, Andhra Pradesh, India. This hatchery is well designed, equipped and maintained for commercial production of *P. monodon* for the last ten years. There are four production units operating simultaneously and the annual production is around 200 - 300 million seeds.

2.3 Collection and treatment of water:

The seawater for the hatchery was pumped from the sea directly using a 7 HP motor. The suction point is located about 20 mt from the shoreline. The water was initially pumped into a sand-gravel filter. From the sand filter, water was lifted into chlorination tank using a 5 HP motor. Chlorination was done with 20ppm chlorine. After 24 hrs, the chlorinated water was stored in overhead tank after passing through activated carbon filter. Subsequently the filtered water was passed through cartridge filter (0.5–1.0: m mesh size) and UV filter before filling into tanks. The residual chlorine available in the treated seawater was determined with chlorine test kits by using O-tolidine. After knowing the availability of excess chlorine in treating sea water sodium thiosulphate (hypo) was used to neutralize the residual chlorine. The chelating agent, EDTA (10ppm) was added in treating seawater to ensure clear sea water. A 15HP air blower and a 7.5HP standby provided a continuous supply of air. The air generated by the blower was supplied with individual tanks through PVC pipes. Samples were tested by PCR to detect the presence of WSSV and MBV. Only negative brooders were stocked in brooder maintenance tanks and fed with polychaete worms and squid at twice in a day. Fifty percent of the water was exchanged daily. The brooders were then transferred to the maturation tanks and treated with water probiotics (Provac) of 20ppm to control the luminous bacteria.

2.4 Design of the Experiment:

Before stocking of nauplii, the larval rearing tanks were first filled with 2 t of chlorinated seawater (30ppt) and all the water quality parameters were checked. Total of 16 tanks, eight for *P. monodon* are controlled tanks where no probiotic was used and another four represents probiotic treated tanks for each species. The water probiotics namely Provac and Microzyme BS were added in the experimental tanks alone at 5ppm each. In addition to probiotics 0.05 ppm of Teflon was also added both experimental and control tanks to prevent the fungal disease.

2.5 Conversions of hatching nauplii:

The newly hatched nauplii from the hatching tank was harvested and transferred in polythene bags and stocked in control and experimental tanks at 2 lakhs per tank. Twenty four hours after stocking, the nauplii converted into zoea – I in experimental tanks. Where as in the control tanks, the conversion time was extended to 24 to 30hrs. First feeding was started when the zoea I appeared. The zoeal stages (I to III) were fed with *Chaetocerossp*. At $1x10^6$ cells /ml in twice daily both control and experimental tanks. The mysis stages (I to III) were fed with algae at density of 5×10^4 cells/ml in both control and experimental tanks for both the species. In addition to algae, mysis stages were fed with knock *Artemia* (*Artemia* killed by hot water) at 5 to 10g/tanks.

2.6 Control of feeding in Artemia:

Post larval stages were fed exclusively on freshly hatched live *Artemia* nauplii at 5 nos per PL per feeding. The water probiotics, Proact (Matrix Biosciences Ltd.) was added daily at 5-10ppm from zoeal stage onwards and at 15 ppm was added after the appearance of post larval stage in experimental tanks alone.

Stock cultures of *Chaetoceros Sp* were maintained at 20-24 ºC with 2000 – 5000 lux light intensity. Conway Walney's Medium or Guillard (F2) Medium was used for indoor culture. Culturing of *Chaetoceros* sp. in 2 litre glass bottles, 25 litre plastic bags, 500 litre FRP tanks and 20 – 30t cement outdoor tanks did scaling up of culture. For outdoor culture, TMRL and Skelon media were used. Once the algal culture reached into exponential phase and sufficient cell concentration was pumped into larval rearing tanks.

The commercial *Artemia cysts* were aerated for half an hour prior to de-capsulation. Two litres of liquid chlorine and 120 ml of Sodium hydroxide solution were mixed well. Cysts were transferred to this solution with constant stirring below 40ºC. The colour changed from dark brown to orange indicates the cyst underwent de-capsulation. At this stage, cysts were transferred to *Artemia* hatching tanks after thorough washing in fresh water until the chlorine smell disappears. Continuous aeration and illumination with a 60V lamp was provided to accelerate the hatching process. After 24hrs, using phototactic behaviour of the Atemia nauplii collected hatched nauplii.

Water exchange was done from the mysis III stage onwards. Using mesh size of 0.5 mm reduced around 50% of the water. Once post larvae appeared, the salinity was reduced slowly and maintained to 20ppt especially in experimental tanks, because, the effect of probiotics was well in low salinity.

2.7 Measurement of the water quality:

The quality of the water parameters of the probiotics treated and control tanks were regularly monitored. Water quality parameters such as salinity, temperature, pH, dissolved oxygen, ammonia, Hardness and alkalinity were estimated daily in the morning hours. The water salinity was measured by using a hand refrectometer (Erma-Japan). The pH of the water was measured by using electronic pH pen manufactured by the Hanna Instrumental Company, Japan.

Dissolved oxygen was estimated by A113 Dissolved Oxygen Bench top Meters. First using sodium bicarbonate after standardized the sulfuric acid then the samples were titrated with the standardized sulfuric acid by using the methyl red indicator. Ammonia level was monitored regularly by adopting the method (12).

2.8 Challenging for WSSV:

The WSSV filtrate solution was prepared following the method

of Chou *et al.,* (1995) (13) with minor modifications. Shell and attached epidermis were collected from frozen (-80°C) black tiger shrimp from a shrimp culture pond that had been infected with WSSV. The tissues were homogenized in saline water (1:9 v/v) at 4°C. After centrifugation at 17 345 X g for 5 min, the supernatant fluid was filtered through a 0.45 pm membrane. The filtrate was used as the WSSV stock filtrate solution. In a series of immersion and injection trials, the optimal challenge dilution levels (48 hr of the WSSV stock filtrate was estimated as 500x for immersion of post larvae (14).

2.9 Study on the field trials:

The farm: The farm selected for the present study was situated at a Village by Name Kudithipalem 20 kilometer away from Nellore city and just nearer to coast of Bay of Bengal. The total farm consist of one division (A) for separate culture of *P. monodon* Division A is used for farming for *P. monodon* culture farming. Each division consist of 5 ponds (2 Reservoirs, 2 Control ponds, A1-A3) of each of 0.5 hectare size of which three are used for farming shrimp with probiotics (A1-A3 for *P. monodon*) and another one as control (A4 for *P. monodon*) without the application of probiotics. One pond from each division acts as a reservoir. In the first step of initiation of culture all the ponds were checked for pH and those which reported less than 7.5 were treated with required amount of lime. After three days the ponds were filled with chlorination treated reservoir water. The water quality of all the ponds was checked with standard protocols (APHA standards). For the proper development of bloom 50 kg of deoiled rice bran along with 20kg of urea per pond was applied. Now these ponds are ready for seeding.

Probiotics selected: Two probiotics were selected for the present study to assess the effect of them in farming shrimp. They are Provac, a water probiotic from Matrix India Ltd and a feed probiotic Gut act from Salem microbes, Chennai. Both the formulations are rich in lacto bacillus.

Seeding: *P. monodon* produced in the Lila hatchery by using probiotics as described above were procured in double layered polythene bags with 1/3 water and 2/3 rd oxygen with 500 pulses per bag in a fully air-conditioned vehicle. After reaching farm the seed bags were transferred to pond of their respective, and left them for about an hour to match the conditions of both pond and seed bags. Each pond of a series was seeded with *P. monodon* Pls (75,000 Pls /pond).

Feeding and application of probiotics: All the seeds were fed with commercial feed (CP Ltd). For ponds A1 only water probiotic was applied and the animals in the ponds A2 was fed with a gut probiotic mixed feed whereas ponds A3 received both gut and water probiotics. The Control ponds A4 were maintained without probiotic treatment. The dosage was given in the table-

Water quality monitoring: The water quality parameters were monitored regularly in both control and treated ponds. The water level was measured by using a standard scale with centimeter marking. The water salinity was measured using a hand refractometer (Earma, Japan) and the pH was measured using a digital pH meter (Elico Ltd). The total alkalinity, hardness and ammonia levels were estimated as per the standard protocols (APHA 2010). The water temperature was measured in the pond itself using a thermometer. Dissolved oxygen meter was used to estimate the dissolved oxygen levels. Transparency was measured in terms of light penetration using a search disc.

Growth measurement: Cast net was used to measure the growth rate of the sample. The first sampling was taken after the 40th day and number of individuals and average body weight (ABW) was measured. Sampling was regularly performed after every 10 days not only to asses the growth but also to Check the healthiness of animals (15).

Microbial Analysis: For microbial analysis the water and sediment samples were collected separately from different parts of the ponds in sterile conical flasks and were mixed to make a single sample. This process was repeated for every pond and final samples were subjected to analysis. For an enumeration of heterotrophic bacteria, the Zobells Marine agar was used and for vibrio isolation TCBS agar was used as media. After inoculation the plates were incubated at 29^{0}C for 24hrs and colonies were counted using a digital colony counter. Luminescent bacteria were identified by observing plates in dark room. At the end of the experiment, the individuals were counted in determining the rate of survival in each tank and weight was recorded for growth studies then, the individuals were sacrificed for proximate and biochemical analysis.

3.0 RESULTS AND DISCUSSION:

The use of probiotics in foods has become increasingly popular since these live and/or dead bacterial preparations, when consumed in adequate quantities, may confer health benefits on the host (16). All the ten commercial probiotics selected were screened to the heteroptriopphic compositions. All selected probiotics were shown to have 109 cfu/ml on their labels but the results have reported that except probiotic 7 all other are having the lower counts than represented. In some of the products (3 and 4) the counts were very low. In one probiotic sample (Probiotic 10) the label does not have any information regarding the heterotrophic counts.

The effectiveness of probiotics was also estimated by their survivalance under different pH particularly under acidic conditions. The survival percentage in all most all probiotics was normal ranging from 76 to 89. The percentage is very high and is more than 95 % in two probiotics i.e in probiotic 2 and 7. Approximately the same percentage was maintained not less than 90 at all incubation periods. The survival percentage in remaining probiotic solutions has decreased considerably as the incubation time increases from 1hr to 3hrs (Table 2).

Commercial probiotic	0.3%(240 min)	0.5%(240 min)	1%(240 min)
Product 1	96+/-2.9	95+/-1.9	93+/-2.1
Product 2	98+/-1.0	97+/-1.1	96+/-1.4
Product 3	88+/-5.2	81+/-4.2	78+/-4.2
Product 4	94+/-3.9	91+/-3.6	90+/-3.0
Product 5	97+/-4.2	90+/-3.2	87+/-1.0
Product 6	89+/-3.9	82+/-3.9	80+/-3.9
Product 7	98+/-1.7	94+/-1.7	93+/-1.7
Product 8	86+/-2.9	80+/-2.9	76+/-2.1
Product 9	89+/-4.4	88+/-4.4	81+/-2.4
Product 10	89+/-4.2	87+/-4.2	87+/-1.2

Table 2. Survival rate under different concentrations of oxgall treatment

3.1 Disease prevalence in Post larvae under probiotic treatment:

The general appearance under microscope the post larvae were taken on a slide and were observed under a microscope. Both post larvae were clean in appearance with clear chromatophores which are at their stage-II as dots which indicates that there no stress on the post larvae. The muscle gut ratio in almost all the post larvae observed was 1:4. Virtually no necrosis of muscle or the appendages is seen in both test groups of post larvae. A slight

necrosis were observed under low dose (10 and 15ppm) treatments of probiotics with *P. monodon* post larvae whereas the control tank post larvae were seen with considerable necrosis. The *P. monodon* post larvae were tested for viral infections like Monodon baculo virus (MBV), Hepato pancreatic parvo virus (HPV) and white spot syndrome virus (WSSV) and the results were negative for both controls and treated tanks whereas the *P. vannamei* post larvae were tested only for WSSV through PCR using the kit supplied from IQ 2000 and was also negative for both control and treated tanks (Table-3).

WSSV	HPV	MBV	Necrosis	Control	Treated	Necrosis	MBV	HPV	WSSV
-	-	-	++++	MC1	MT1	++	-	-	-
-	-	-	+++	MC2	MT2	+	-	-	-
-	-	-	+++	MC3	MT3	-	-	-	-
-	-	-	++++	MC4	MT4	-	-	-	-

Table. 3: Disease prevalence in *P.monodon* post larvae between probiotic treated and control tanks

3.2 Water quality maintenance:

3.2.1. In P. monodon tanks:

Proper maintenance of water quality in hatchery tanks is of utmost important factor for the better production of post larvae. At each stage of development post larvae starting from egg to post larvae the water quality was monitored. Water quality parameters like pH, salinity, Alkalinity, total hardness, ammonia and dissolved oxygen levels were regularly measured and a record was maintained. The average values of all parameters in each tank was tabulated (Table 4). The pH was varied in probiotic treated ponds from 7.8 to 8.2 and that of controls the value remained below 8. The salinity was similarly maintained in both control and treated ponds i.e 20ppt. The average total alkalinity levels were also varied from 159 ppm to 188 ppm in probiotic treatment tanks where as in control tanks the average values varied from 177ppm to 189ppm. The average hardness values were measured by present of total of calcium and magnesium in water. The values were changed from 4900 to 5450 p.m. in control ponds whereas in treating ponds the total hardness had been elevated above 5000ppm always. The most important factor among the water quality parameters is the dissolved oxygen which was at considerable levels in controls and as well as in treated ponds (not less than 3 ppm). In comparison the values were higher for probiotic treated tanks than control tanks. The average DO levels in treated ponds were recorded between 4.09 to 4.97 ppm. The ammonia levels also not varied much in the control as well as in treated tanks i.e 0.4ppm in controls and 0.3 ppm in that of probiotic treated tanks (Table 4).

3.2.2. P. monodon post larvae:

In the present study we have also estimated the levels of anti-oxidant enzyme levels produced in response to probiotic treatments against ROs generated. The SOD levels were not much varied in control tanks whereas there is considerable variation in probiotic treated post larvae the levels of Sod was found to increase gradually with the concentration of probiotics applied. The value has increased from 12.27 to 21.63. The same type increased was also seen with the CAT value also where the increase in probiotic treated tanks varied from 34.12 to 49.55. While assessing immune status the PO levels were observed and was found to increase with concentrations of probiotic and in particular the high values were reported for the last two concentrations (15 to 20ppm) (Table 4).

Pond No	SOD		CAT		PO	
	Control	Treated	Control	Treated	Control	Treated
1	10.21	12.27	32.02	34.12	0.19	0.16
2	10.33	15.23	35.32	39.02	0.26	0.21
3	12.44	17.24	32.00	42.42	0.26	0.29
4	11.56	21.63	37.72	49.55	0.21	0.31

Table. 4: Comparison of Antioxidant status in *P.monodon* post larvae between treated and control tanks.

3.3. Water quality maintenance:

3.3.1. Temperature Cultured tanks of P.monodon:

The water quality in control as well as probiotic treated plant was monitored weekly and represented as an average for the total period of culture. The parameters that were monitored include pH, salinity, Alkalinity, hardness and ammonia along with the temperature.

The temperature in control pond (M1) was 29^{0}C on an average of for four months of culture. It was never increased over 30^{0}C in the midterms and the average temperatures of for the treated tanks were recorded as 28, 29 and 27^{0}C for water probiotic treated, feed probiotic treated and both water and feed probiotic supplemented ponds respectively.

Parameter Tank No	pH	Salinity (ppt)	Alkalinity (ppm)	Hardness (ppm)	Ammonia (ppm)	DO (Ppm)
MC1	7.2	20	177	5300	0.10	3.14
MC2	7.4	20	170	5100	0.11	4.02
MC3	7.8	20	189	5450	0.06	4.10
MC4	7.5	20	189	4900	0.09	3.45
MT1	7.8	20	159	5100	0.02	4.50
MT2	7.9	20	174	5270	0.05	4.97
MT3	8.2	20	166	5230	0.02	4.09
MT4	8.2	20	188	5020	0.03	4.19

Table. 5: Comparison of Water quality between treated and control tanks of *P. monodon*

3.3.2. Microbial counts:

Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganism. The microbiota in the gastrointestinal tract of aquatic animals can be modified by ingestion of other microorganisms. Therefore, microbial manipulation constitutes a viable tool to reduce (or) eliminate the incidence of opportunistic pathogens (17).

Hence it is very much in need to assess the growth of gut microbial floral count. In the present study we have estimated the number of different bacteria flora present in the gut of both *P. monodon* from the pond M4 and V4 at 60 DOC and 120 DOC respectively. The total plate count in control pond is much higher when compared to probiotic treated ponds in both monodon and vannamei cultured ponds. In control ponds M1 and V1 it was about $5.6x10^8$ and$5.2x10^8$and the count was decreased enormously in treated ponds to $2.5x10^8$clls/g in M4 and $2.3x10^8$ cells/gm in V4 respectively after 60DOC and the count was found to further decreased to $2.2x10^8$ in both ponds after 120 DOC.

The total color form number also decreased in both the vannamei and monodon probiotic treated ponds as the days of culture increased. The count was found to be low in treating ponds i.e. in M4 and V4 at 120 days of culture whose count was decreased from 1586 (M1) to 1034 (M4). The same condition was also seen with cultured ponds where the count has decreased from 1486 (V1) to 1232 (V4) after 60 days and further decrease to 1114 cells/gm was recorded after 120 days of culture. The total fecal coli forms and facultative anaerobes count was also much reduced than the control pond counts. But the lactobacillus count

was found to be increased than the control. The total lactobacillus that were reported from the gut of control ponds shrimp were $1.7x10^6$ cell/ml (M1) and $1.6x10^6$ cells/ml (M2) respectively. This value has much increased to $4.9x10^7$ and $4.7x10^7$ cells/ml respectively in M4 and V4 ponds after 120 days of culture. The replacement of bad and infectious bacteria like coli forms and facultative anaerobes by useful bacteria like bacillus indicated the good status of digestion and less possibility of infection of the animal.

Pond No	Yellow Colonies				Green colonies				Luminescent bacteria			
	30 DOC	60 DOC	90 DOC	120 DOC	30 DOC	60 DOC	90 DOC	120 DOC	30 DOC	60 DOC	90 DOC	120 DOC
M1Con	300	320	210	190	100	90	90	120	30	Nil	Nil	Nil
M2	270	270	180	180	60	60	70	70	10	Nil	Nil	Nil
M3	290	280	220	240	10	NIL	Nil	10	Nil	Nil	Nil	Nil
M4	260	240	170	150	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

The most important bacteria that generally affect the shrimp farming is *Vibrio* species. The number of bacteria/ml is a very important factor to be studied. The vibrio counts were estimated regularly and reported average number for every 30 days of culture in all control and treated ponds of both *P. monodon*. In the *P. monodon* control tank the number of *vibrio* forming yellow colonies were found to decrease gradually from day 1 to end of the culture where as a reverse was seen the green colony forming bacteria where the number has increased from 100 cfu/ml to 120 cfu/ml from 30 DOC to 120 DOC. But in treating ponds of Monod on the number of yellow colonies were found to decrease uniformly in all ponds (in M2, M3 and M4). The green colonies were reduced to 60-70cfu/ml in the water probiotic treated pond during the culture and it was reported nil in M3 and M4.

3.4 Growth patterns:

The effect of Water Probiotics and Feed Probiotics on the growth patterns of *P. monodon* during culture activity. In the present study the growth patterns of both shrimp were monitored in different conditions i.e. Control condition, where only feed was provided, in another condition along with normal broadcasting of feed water probiotics were used to keep the water environment clean, in another condition along with normal feed, feed probiotics were mixed broadcasted and in the Fourth condition both feed probiotics and water probiotics were introduced in the cultural environment. The following are the four regimes.

- Control (Feed broadcasted)
- Normal Feed + Water Probiotics used
- Normal Feed + Probiotic Feed used
- Feed Probiotic + Water Probiotics used

The growth patterns of *P. monodon* were monitored in the above conditions mentioned and presented Table. The growth patterns were recorded for one 120 days on a monthly basis, starting from January to April. The growth patterns recorded for control *P. monodon* clearly demonstrates that, the animals obtained 30.12 hm g maximum weight during the 120 day culture period. In the control, the culture conditions include, the normal feeding activity without any addition of either Water Probiotics or Feed Probiotics. In the second condition, where Water Probiotics are regularly used to clean the water environment along with normal feeding activity, the growth patterns were also shown relatively good progress obtaining 31.87 g of average weight at the end of culture activity. In another set of experiments Feed Probiotics are mixed with Feed and subsequently broadcasted in the cultural environment, the prawns showing better growth rates and obtaining 33.06 g of average weight at the end of culture activity compared to the control weight of 30.12 g. In the last set of experiment both water probiotics and Feed Probiotics were used and the growth patterns are recorded to be maximized and the shrimps obtained an average weight of 34.07 g compared to control weight. In the Control conditions the maximum weight obtained was 31.12 g compared to 33.87 g obtained in Water Probiotics used ponds, 35.06 g in the Feed Probiotics used ponds and 36.07 g in the feed and Water Probiotics used ponds (Table 7).

Month	Control (M1)	With Water Probiotic (M2)	With Feed Probiotic (M3)	With WP+FP (M4)
January	3.12	4.31	4.6	5.01
February	7.88	8.87	9.23	10.2
March	16.94	18.88	19.36	20.18
April	26.12	28.87	29.06	34.07

Table. 7: Growth of *P. monodon* juveniles under different probiotic treatments.

The use of probiotics or beneficial bacteria, which control pathogens and environmental pollution in aquaculture through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment. The use of probiotics in human and animal nutrition is well documented (22, 23, 24) Fuller, 1992; Mulder *et al.,* 1997; Rinkinen *et al.,* 2003) and recently, they have begun to be applied in aquaculture (26 27, 28, 29, 30).

l was reported for seven probiotics (1, 2, 3, 5, 7, 9 ,10 and 25) of which the 7th probiotic sample have shown a higher assimilation of 27ug/ml. The minimum assimilation capabilities were recorded for sample 4 (14ug/ml) and 6 (16ug/ml) respectively (Table 5). β-Galactosidase activity and cholesterol assimilation are important features for strains intended for use as probiotics. Four isolated *L. bacillus* species of this study produced a high activity of β-galactosidase (Table 3), as reported earlier for other *L. plantarum* strains (38, 39, and 40). β-Galactosidase can hydrolyze lactose to form glucose and galactose, alleviating lactose intolerance that exists in large proportion of the world population. High concentration of cholesterol in the blood streams of humans has been recognized as a risk factor in the coronary heart disease. Consumption of fermented milk products containing certain lactobacilli or bifidobacteria has been claimed to decrease the concentration of the blood stream cholesterol in humans (41, 42, 43, and 44). The present study showed that all the isolated *Lactobacillus* species were able to assimilate cholesterol in MRS medium. However, further tests on animals or humans should be done to prove whether these strains could reduce serum cholesterol level *in vivo* (36).

Therefore, it is important that the sustainability of this industry is maintained by improved Aquaculture practices coupled with the more effective use of scientifically approved disinfectants and sanitizers as feed supplements along with other biological agents in order to improve survival rates and growth to enhance yield and to minimize production cost.

4.0 CONCLUSION

Research and application of Probiotics in Aquaculture in India is still in its infancy and not much of the commercial Probiotic product was licensed in India so far. It is essential to understand the mechanism of action in order to define selection criteria for potential probiotics. Therefore more information on the host/microbe interactions *in vivo* and development of monitoring tools are still needed for better understanding of the composition and functions of the indigenous micro biota as well as of microbial cultures of 'Probiotics'.

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